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Prostate cancer is the most common cancer in American men. It is also characterized by a substantial racial/ethnic variation in risk: highest in African-American men, lowest in Asian men and intermediate in Caucasian and Latino men. We propose to investigate genetic variants of genes involved in the regulation of prostatic growth and particularly in androgen metabolism, particularly the HSD3B2 gene which encodes the type II β-hydoxysteroid dehydrogenase. Our current progress is highlighted by the following three findings. First, our data indicate that the locus under investigation is highly polymorphic in constitutional DNA. Furthermore, our population-based investigations are proceeding according to plan with MAPA technology. Finally, our initial biochemical results suggest that this aim will also be completed soon.

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Table of Contents

PROGRESS REPORT

Juergen K. V. Reichardt, PhD

Abstract

Prostate cancer is the most common cancer in American men. It is also characterized by a substantial racial/ethnic variation in risk: highest in African-American men, lowest in Asian men and intermediate in Caucasian and Latino men. We propose to investigate genetic variants of genes involved in the regulation of prostatic growth and particularly in androgen metabolism, particularly the HSD3B2 gene which encodes the type II β-hydoxysteroid dehydrogenase. Our current progress is highlighted by the following three findings. First, our data indicate that the locus under investigation is highly polymorphic in constitutional DNA. Furthermore, our population-based investigations are proceeding according to plan with MAPA technology. Finally, our initial biochemical results suggest that this aim will also be completed soon.

Overview

This proposal is part of a research program aimed at identifying genes involved in the predisposition to and progression of prostate cancer among various racial/ethnic groups in the US. Prostate cancer will be diagnosed in 220,900 men in the US in the year 2003 alone. Some 28,900 individuals die of this disease annually. Prostate cancer is characterized by substantial racial/ethnic variation in risk: highest in African-American men, lowest in Asian men and intermediate in Caucasian and Latino men. We proposed to investigate as our central hypothesis that genetic variants of genes involved in the regulation of prostatic growth and particularly in androgen metabolism by themselves and in combination significantly contribute to prostate cancer risk and progression. Specifically, we proposed to examine the hypothesis that DNA sequence variations in the type II 3β-hydoxysteroid dehydrogenase (HSD3B2) gene contribute substantially to the risk of prostate cancer particularly across racial/ethnic lines. The "candidate gene", HSD3B2, was chosen because the reaction substrate [i.e. dihydrotestosterone (DHT)] of the enzyme encoded by this gene modulates directly cell division in the prostate. Epidemiologic evidence suggests that variation in DHT levels play an important role in risk of prostate cancer. Thus, 3β-hydoxysteroid dehydrogenase activity encoded by HSD3B2 variant alleles may be important in regulating intraprostatic DHT steady state levels by controlling its degradation. This candidate gene encodes the enzyme that initiates the irreversible inactivation of DHT.

Specifically, in this project we proposed to test, using a case-control study approach within a multi-ethnic cohort study design, the association between prostate cancer risk and its progression and HSD3B2 allelic variants among four major racial-ethnic groups. Our original three interrelated specific aims were:

- To identify all allelic variants in the HSD3B2 locus by sequencing 200 men from four racial/ethnic groups (African-American, Japanese-American, Latino and Americans of European ancestry (Caucasian) men).

- To determine the relationship between the HSD3B2 gene and prostate cancer by genotyping polymorphic DNA markers in the HSD3B2 gene in up to 800 men with prostate cancer and controls from four racial/ethnic groups who are at very different risks of prostate cancer.
- To determine the *in vitro* biochemical properties of HSD3B2 variants identified in specific aims 1 and 2.

Progress Report

Pursuant to specific aim 1, we have sequenced in constitutional ("germline") DNA of 120 men with the following make-up: 30 from each of the four racial/ethnic groups (African-American, Asian-(Japanese-)American, Caucasian and Latino, the entire HSD3B2 gene. Half are prostate cancer cases, half are controls. Our sequencing has identified 17 additional polymorphisms that are highlighted above the gene in Fig. 1.

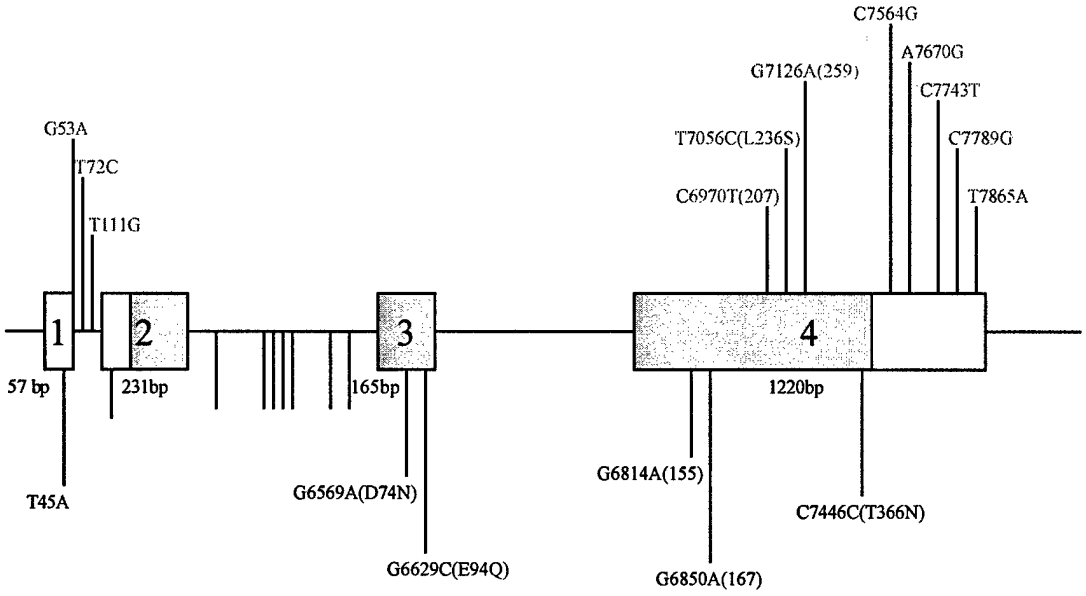

Figure 1: Newly discovered constitutional ("germline") SNPs in the HSD3B2 gene.

In short, the HSD3B2 gene in humans is highly polymorphic and our work has uncovered significant unreported additional genetic variation. This specific aim is complete as of this year.

In order to advance specific aim 2 we have begun genotyping 868 men for the D74N, E94Q, L236S and T366N constitutional DNA polymorphism that change amino acids and the T1362G SNP (cf. Fig. 1). Sample genotyping using a multiplex approach

(MAPA: Multiplex Automated Primer Extension Analysis; developed in this lab) on an ABI3100 instrument is shown in Fig. 2. The genotyping is about 2/3 complete since we genotyped 642 of 998 samples. All MAPA genotyping will be completed shortly and certainly by the end of this grant. The data analysis phase will then begin and investigate associations as appropriate.

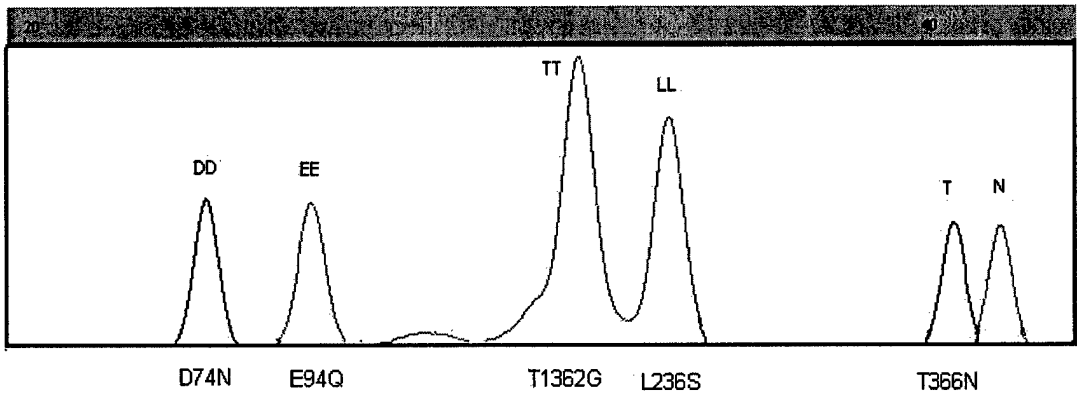

Figure 2: Sample MAPA genotyping of the HSD3B2 gene on an ABI3100 automated sequencer. Only the last, T366N, SNP is polymorphic (heterozygous) in this sample.

We have made progress in specific aim 3 on the *in vitro* biochemistry on several fronts. We have reconstructed all five missense SNPs in constitutional DNA (cf. Fig. 1) and plan to assay them soon. We have begun preliminary *in vitro* experiments and will then assay all mutants. We expect to make significant progress in the final year in this area.

Benchmarks 2003:

Specific aim 1: Complete.
Specific aim 2: Genotyping work is ~2/3 complete. Data analysis to follow in the final year.
Specific aim 3: Mutagenesis is complete. Assays to proceed in final year.

Addendum 10/03

Key Research Accomplishments

- 18 SNPs identified in constitutional DNA in the HSD3B2 gene.
- MAPA genotyping for 5 SNPs in the HSD3B2 gene is set up and ongoing.
- 5 SNPs are reconstructed in the HSD3B2 cDNA for *in vitro* analyses.

Reportable Outcomes

None to date.

Conclusions

Significant progress toward all three specific aims was made in the past year. We plan to complete all three specific aims in the coming 12 months.

Our research may result in better presymtomatic diagnosis of prostate cancer and will lead to a better fundamental understanding of the HSD3B2 gene and its enzyme.